Phosphorous, All Forms (Colorimetric, Ascorbic Acid, Single Reagent)

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Revision Record

Revision No.	Date	Responsable Person	Description of Change
9	12/27/11	QA Officer	Previous Release
10	09/03/13	QA Officer	Added electronic signature and revision record requirements. Revised SOP Identification number.
11	12/9/15	Technical Director	Minor grammatical errors corrected.
12	8/11/16	Technical Director	Additional step to sample analysis procedure.
13	02/21/2018	Technical Director	Correction for new instrument and standards. Updated batch quality, data recording, and records sections.
14	01/10/19	Wet Chemistry Department Supervisor	Calibration procedure reflects monthly calibration for Total Phosphorus analysis.

REFERENCES:
- EPA Method 365.2
- Standard methods, 18 edition, Method 4500-P-A,B,E
- Hach Method 8190

SAFETY
Lab coat, gloves, and safety glasses with side shields should be worn at all times.

HOLDING TIME & SAMPLE RECEIPT

Orthophosphate: glass or plastic, non-preserved. Hold at 4^{o}C; analyze within 48 hrs.
Dissolved Phosphorous: glass or plastic, non-preserved. Hold at 4^{o}C; filter and preserve with sulfuric acid to a pH <2 immediately upon laboratory receipt; analyze within 28 days.
Total Phosphorous: Preserve with H_2SO_4 <2, glass or plastic. Hold at 4^{o}C; analyze within 28 days.

GENERAL

Ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the phosphorus concentration.

Only orthophosphate forms a blue color in this test. Polyphosphates (and some organic phosphorus compounds) may be converted to the orthophosphate form by sulfuric acid hydrolysis. Organic phosphorus compounds may be converted to the orthophosphate form by persulfate digestion.

INTERFERENCES:

- High iron concentrations can cause precipitation of and subsequent loss of phosphorous.
- There are high concentrations of phosphorous in most detergents; therefore soaps should not be used to clean the glassware. All glassware must be soaked in an acid solution for a minimum of 30 minutes prior to use, then rinsed with DI and acid washed thoroughly.
- Arsenate is determined similarly to phosphorous and should be considered when present in concentrations higher than phosphorous. However, at concentrations found in seawater, it does not interfere.

APPARATUS, REAGENTS & STANDARDS:

Apparatus

- Erlenmeyer Flasks 125mL
- Disposable 200mL Beakers
- Class A 50mL graduated cylinder
- Class A volumetric flasks 100, 1000mL
- Class A volumetric pipettes 1, 2, 5, and 10mL
- Finnpipette automated pipettes, 50-200uL and 200-1000uL
- Spectrophotometer, HACH Odyssey Spectrophotometer, DR/3900 at 880 nm
- COD reactor
- Glass microfiber filters, 0.45µm

Reagents

- Sulfuric Acid Solution, 5N: Dilute 70mL conc. H_2SO_4 with DI to 500mL with DI.
- Antimony Potassium Tartrate, 0.2743%: Purchased from RICCA, Cat# 5872-16.
- Ammonium Molybdate Solution: Dissolve 20 g $(NH_4)_6\ Mo_7O_{24}{\cdot}4H_2O$ in 500 mL of DI. Store in a plastic bottle at 4^oC.

- Ascorbic Acid, 0.1M: Dissolve 1.76 g of ascorbic acid in 100 mL DI. This solution is stable for 1 week if stored at 4^oC.
- Combined Reagent: Mix the above reagents in the following proportions for 100 mL of mixed reagent: 50 mL of 5N H_2SO_4, 5 mL of antimony potassium tartrate solution, 15 mL of ammonium molybdate solution, and 30 mL of ascorbic acid solution. Make this standard daily.
- Sulfuric Acid (H_2SO_4), 11N: Slowly add 310 mL conc. H_2SO_4 to 600 mL DI when cool, dilute to 1 liter with DI water.
- Ammonium Persulfate, reagent grade.
- HACH Total and Acid Hydrolyzable Phosphorus Reagent Set: Cat # 27427-45.
- PhosVer Reagent: Dissolve a 1:1 ratio of HACH PhosVer raw chemical and DI water in a volumetric flask. Filter using 0.45µm. Make fresh daily.

Standards

- Stock Orthophosphate Solution (TV = 1000ppm): Purchased from Absolute Standards, part #54105.
- 2^{nd} Source Orthophosphate LCS/MS Stock Solution (TV = 1000ppm): Purchased from Absolute Standards, part #54105, **different lots**.
- Ortho-Phosphate LCS/MS Solution, (TV = 50mg/L): Dilute 5mL of Absolute Standard up to 100 mL with DI.
- Ortho-Phosphate LCS (TV = 0.5 mg/L): Bring 0.25 mL of LCS/MS solution up to 25 mL with DI.
- Primary Source Total Phosphorus (TV = 1000ppm): Purchased from Absolute Standards, part# 54133.
- Second Source Phosphorous LCS/MS Solution (TV = 50 mg/L): Dilute 5mL of Absolute Standard (part #54133, must be **different lot**) up to 100 mL with DI.
- Ortho-Phosphate LCS (TV = 0.5 mg/L): Bring 0.25 mL of LCS/MS solution up to 25 mL with DI.

PROCEDURE:

Analyte Set-Up in HACH Odyssey Spectrophotometer, DR/3900

Touch: User Programs → Program Options → New Program and OK → Program # (next consecutive number) and OK → Set Name as analyte and the last two digits of the year (i.e. OPHOS18 or TPHOS) and Next → Select Single Wavelength, set as 880nm and Next → Select 0.001 for Calibration Resolution and press Cancel → Select Upper Limit and Type in 2.0 → Store

Calibration curve for Total Phosphorus is conducted on a monthly basis, thus the name of the analysis in the Hach Spec does not contain a year.

Curve for Ortho-phos

- Dilute 10 mL of the Stock Phosphate Solution for Curve to 1000mL using DI in a volumetric flask, TV = 10mg/L. Using this primary dilution (PD), prepare the calibration standards using the following dilutions:

Standard, mg/L	Dilution
0.00	100 mL DI
0.02	2 mL of PD to 1000 mL with DI
0.10	1 mL of PD to 100 mL with DI
0.20	2 mL of PD to 100 mL with DI
0.40	4 mL of PD to 100 mL with DI
0.80	8 mL of PD to 100 mL with DI
1.00	10 mL of PD to 100 mL with DI

Curve for Total and Dissolved Phosphorous in Aqueous Samples

- Dilute 1mL Primary Source Total Phosphorus (1000ppm) to 100mL using DI in a volumetric flask. Using this primary dilution (PD), prepare the calibration standards using the following dilution:

Standard, mg/L	Dilution
0.00	100 mL DI
0.02	2 mL of PD to 1000 mL with DI
0.10	1 mL of PD to 100 mL with DI
0.50	5 mL of PD to 100 mL with DI
1.00	10 mL of PD to 100 mL with DI
1.50	15 mL of PD to 100 mL with DI
2.00	20 mL of PD to 100 mL with DI

Curve for Total Phosphorous in Soils/Solids

- Dilute 1mL Primary Source Total Phosphorus (1000ppm) to 100mL using DI in a volumetric flask. Using this primary dilution (PD), prepare the calibration standards using the following dilution:

Standard, mg/L	Dilution
0.00	100 mL DI
0.02	2 mL of PD to 1000 mL with DI
0.10	1 mL of PD to 100 mL with DI
0.20	2 mL of PD to 100 mL with DI
0.40	4 mL of PD to 100 mL with DI
0.80	8 mL of PD to 100 mL with DI
1.00	10 mL of PD to 100 mL with DI

Colorimetric Determination

Orthophosphate

- Mix sample thoroughly and pour 25mL of sample into a vial.
- Treat LCS/MS and blank the same as samples.
    - Blank 25 mL DI.
    - LCS (TV=0.5 mg/L): Bring 0.25 ml of LCS solution up to 25 ml with DI.
    - MS (TV of spike = 0.5 mg/L): Bring 0.25 ml of LCS solution up to 25 ml with sample.
- For dissolved orthophosphate filter through 0.45µm filter.
- Add 4.0 mL of combined reagent to sample and mix thoroughly.
- Clean the outside of the tube with a towel and place in cell holder.
- While the color development is occurring, zero the instrument with a DI water blank.
- After a Minimum of 10 minutes (but no longer than 30 minutes), measure the color absorbance at 880nm for the colorimetric reference blank, the LCS and all samples.
- Results will be read in mg/L.

Total Phosphorus for Aqueous Samples

- Preheat COD Reactor.
- Add 5 mL of Standard, Blank, LCS, or Sample to a Test Vial.
- Add the contents of one Potassium Persulfate Powder Pillow for Phosphate to the vial. Cap tightly and invert to mix.
- Place vial in the COD Reactor and time for 30 minutes.
- Take 10 PhosVer Pillow packs and Dissolve in 10mL of DI.
- Use Syringe Filter to filter new PhosVer pillow pack reagent. Make fresh daily.
- Carefully remove the vial from the reactor. Place it in a test tube rack and allow to cool to room temperature.
- Add 2 mL of 1.00 N Sodium Hydroxide Standard Solution to the vial. Cap and mix.
- Add 1mL of filtered PhosVer reagent to each vial. Cap and invert.
- After a minimum of 10 minutes, wipe the outside of the vial with a Kimwipe to remove any smudges or prints. Zero the instrument and measure the color absorbance of the digested blank, LCS and all samples at 880nm. Results will read in mg/L Phosphorus.

Dissolved Phosphorus for Aqueous Samples

- Filter 50ml of sample immediately upon laboratory receipt using 0.45µ glass fiber filters.
- Following filtering, preserve the sample with 1ml of 1:1 sulfuric acid.
- Once sample is filtered and preserved, follow Total Phosphorus procedure for Aqueous samples (above).

Total Phosphorus in Soil/Solid

- Add 2.5g sample and 50 mL DI to a 150 mL Erlenmeyer flask.

- LCS, TV=0.2, use 1 mL of Second Source Phosphorus LCS/Spike solution in 49mL DI.
- Blank 50 mL of DI.

- Add 1 mL of 11 N H_2SO_4 and 0.4 g of ammonium persulfate to the beaker.
- Boil gently on a preheated hot plate until a final volume of about 10 mL is reached. Do not allow the sample to go dry.
- Cool and adjust the pH to 7- 10 by adding 2-3 drops of phenolphthalein and adding 10N NaOH dropwise until a definite pink forms and bring up to 50mL with DI. In between each sample acid wash the graduated cylinder and then rinse with DI.
- If sample is not clear at this point add 2-3 drops of acid and filter.
- Determine total phosphorus as outlined in the orthophosphate procedure above for colorimetric determination.

CLEANING:

All glassware should be rinsed with a dilute acid and then rinse with DI. Prior to use, all glassware must be soaked in a dilute sulfuric acid wash for a minimum of 30 minutes.

TROUBLE SHOOTING:

Problem	Possible Solutions
LCS out of QC limit	Acid wash glassware and reanalyze batch
Blank above reporting limit	Acid wash glassware and reanalyze batch, if blank is still contaminated, rinse glassware with a very weak potassium dichromate solution
Duplicate out of QC limits	Acid wash glassware and reanalyze batch

CALCULATION:

- To calculate mg/l $P\text{-}PO_4$:

$$mg/l = DF*(m\ (ABS) + y)$$

Where:

m = slope of linear regression plot
y = y-intercept of linear regression plot
DF = Dilution Factor

Relative Percent Difference

RPD=[ABS(sample conc.-MD conc.)/((sample conc.+MD conc.)/2)]*100

Matrix Spike Recovery

% Recovery = [(MS Conc. - Sample Conc.)/ Spike Conc.]*100

QUALITY CONTROL LIMITS/PERIODIC EVALUATIONS:

- The digested blank must be run with each batch of samples up to 20 and the result must be below the reporting limit.
- An LCS must be prepared and analyzed each batch up to 20 samples and Recovery must be within 90-110%.
- A duplicate and matrix spike sample must be analyzed for every 20 or fewer samples processed as an analytical batch. Matrix Spike/Duplicate data is useful for the data user to assess if, and to what extent, the sample matrix affects recovery. If the Matrix Spike recovery is 80-120%, it can be generally assumed that little matrix interference is present. Recoveries below 80% do not invalidate batch data, but serve to indicate matrix effects for that particular sample. An RPD of less than 20% is a good indicator that matrix interference did not occur.
- A MDL study is conducted on an on-going basis. Procedure is followed as outlined in the QA Manual.
- Each analyst must perform an annual demonstration of proficiency by bringing 4 standards through the procedure.
- At the end of the batch and every 20 samples, re-analyze the Blank and LCS to verify that the Spectrometer has not drifted during analysis.

DATA RECORDING:

Access handwritten data sheets for Ortho-phosphate or Total Phosphorus on the server: "NASOMV\Wetchem \Analyses\Phosphorus". Record reagent numbers, project case identification numbers, sample identification names, results, volumes, dilutions, analyst, date and time, and all required QC documentation are recorded on data sheets. Upon completion of analysis, batch samples in Promium Element with corresponding date, time, and sample volumes for each sample and select appropriate LCS reagent numbers and volumes. Export data into an auto-populated bench sheet through Element for the specific analysis (i.e. Tphos Excel spreadsheet for Total Phosphorus). Excel files have an auto-generated name based upon analysis, batch number, and date. Transcribe all results. Electronic bench sheets are automatically saved in the corresponding folder.

RECORDS:

Excel files are printed and attached to the handwritten data sheets. Electronic and paper copies are filed in the monthly Phosphorus & COD folder. Handwritten copies are transferred to the wet chemistry section of the records storage building, annually. Records of reagents, standards, etc. are recorded electronically in Promium Element.

DEFINITIONS:

- Total Phosphorous (P): all the phosphorous present in the sample, regardless of the form, as measured by persulfate digestion.
- Total Orthophosphate (P, ortho): inorganic phosphorous [$(PO_4)^{-3}$] in the sample as measured by the direct colorimetric analysis procedure.
- Dissolved Phosphorous (P): only the phosphorous present in the sample which is dissolved into the aqueous phase.
- CCB- Calibration Control Blank
- LCS- Laboratory Control Standard
- MDL- Method Detection Limits
- CCV- Calibration Control Verification
- RPD- Relative Percent Difference
- MD- Matrix Duplicate
- DI Blank - a blank of *JUST* DI water used to zero the spectrophotometer
- Digested Blank - A DI water sample carried through the entire sample preparation process to which color regents has been added. ***MUST*** be treated the same way as the LCS and samples.